The histological score of the 0.24% gradient collagen was the best of all experimental conditions. The significant differences existed between the 0.18% gradient collagen and the 0.24% gradient collagen at 4th and 8th postoperative week ($p<0.05$).
In the 0.24% gradient collagen, type II collagen was strongly and widely stained in both the regions, and type I collagen was weakly stained in the central region.
In vitro haptotactic manner,the 30 times gradient generated the most effective migration activity for the cultured MSCs.
Conclusions: Concentration-gradient collagen scaffold recruits effectively the progenitor cells to the center of full-thickness cartilage defect and enhances regeneration of the full-thickness cartilage defect. *In vitro* migration assay suggest that there is an optimal gradient when using the gradient collagen cylinder to augment regeneration of full-thickness cartilage defect.

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CANCELLOUS BONE MATRIX GELATIN CONTAINING ALLOGENIC CHONDROCYTES FOR THE REPAIR OF FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS: RESULTS OF A 1 YEAR STUDY IN RABBITS

J. Cao[1], B. Yang[1], A. Zhang[1], J. Chen[1], Z. Zhang[1], Q. Fu[1], C.E. Hughes[2], B. Caterson[2]
[1]*Medical School of Xi'an Jiaotong University, Xi'an, China;*
[2]*Cardiff University, Cardiff, United Kingdom*

Purpose: To investigate the effect of using cancellous bone matrix gelatin (BMG) seeded with allogeneic chondrocytes for repairing full-thickness articular cartilage defects in rabbits.
Methods: 1 month old rabbit chondrocytes were isolated and expanded in vitro by monolayer culture. Passage - 1 allogenic chondrocytes were seeded onto prefabricated cancellous BMG scaffolds to construct tissue-engineered cartilage grafts and cultured in vitro for 2 weeks. These grafts were then implanted to repair mature rabbit knee joint osteochondral defects. As a "Control", replicate defects were filled with cancellous BMG only. After 1 year the treated defect and adjacent sites were harvested and evaluated for macroscopic tissue morphology, Haematoxylin and Eosin (HE), Toluidine Blue (TB) and Picricsirius Red staining as well as immunohistochemistry for typeIIcollagen expression.
Results: After 2 weeks, the allogenic chondrocytes attached to the cancellous BMG, formed a cartilagenous tissue graft of 5-8 cell layers on the surface of the BMG and secreted matrix proteoglycans and collagen type II. Following 1 year after the transplant operation, histological staining of the defect site tissue revealed that reparative tissue had integrated well into the host cartilage and reconstructed the damaged subchondral bone plate in all defects where the BMG-allogenic chondrocyte grafts had been implanted. These defects were fully filled with hyaline-like cartilage in this group with cells located in lacuna and arranged in columns in the deeper zones of the repair tissue. Matrix staining showed the presence of proteoglycans and collagen type II evenly distributed and well-integrated within the adjacent articular cartilage. In contrast, in the defects that were filled with BMG scaffold alone, these mainly formed a fibrocartilagenous repair tissue with irregularly distributed chondrocytes and reduced staining for matrix proteoglycans and collagen type II.
Conclusions: Cancellous BMG is an effective scaffold for use in cartilage tissue engineering procedures. Cancellous BMG seeded with allogenic chondrocytes was successful in producing well-integrated hyaline-like cartilage repair tissue after transplantation for 1 year and this method shows great promise for treatment of joint osteochondral defects in human patients.

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CHONDROGENESIS OF EMBRYOID BODIES AND CELLS DERIVED FROM EMBRYOID BODIES

Z. Zhang
Saint Louis University, St. Louis, MO

Purpose: Embryonic stem cells (ESCs) are a promising reparative source for cartilage injury and degenerative joint diseases. Generally, ESCs are differentiated in the form of an embryoid body (EB), a stem cell aggregate contains progenitor cells for all three germ layers of embryo. The EB contains elements of chondrogenesis. Therefore, under a suitable condition, chondrogenesis should occur in EBs. However, most of the current studies involving chondrogenesis of stem cells are using cells that are derived from EBs (EBd cells).
Methods: This study was to investigate, at a similar differentiation stage, whether EBs and EBd cells have similar potentials of chondrogenesis. Mouse embryonic stem cells were cultured in suspension for EB formation. EBs were obtained at day 5 of EB culture. EBd cells were cells from collagenase-dissociated EBs. EBs and EBd cells were centrifuged for pellet formation. Both EB and EBd cell pellets were cultured in chondrogenic medium for 3 weeks. The pellets were sampled at weeks 1, 2 and 3 for histology and immunohistochemistry.
Results: There was a significant amount of cell death in both pellets of EBs and EBd cells after one week in culture. Type II collagen was positively stained in the EBd cell pellets, but not in EB pellets. More type II collagen staining was observed at the peripheral area of the EBd cell pellets. EBd cell pellets were also stained with aggrecan. Both EB and EBd cell pellets were stained with type I collagen.
Conclusions: This study shows that dissociation of EBs is required for chondrogenesis in pellet culture. The dissociation process allows cells to reorganize in the pellets and respond to the chondrogenic medium. The chondrogenic process in chondrogenic medium is also a process of cell selection as EBs and EBd cells are heterogenic cell population. Significant cell death in both types of pellets is evident in that only a fraction of EBd cells have the potential of chondrogenesis. However, this fraction of cells in EBs did not respond to the current chondrogenic condition, indicating the importance of an extracellular microenvironment in stem cell differentiation.

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TRANSPLANTATION OF MESENCHYMAL STEM CELLS TO TREAT EARLY CARTILAGE LESIONS IN EXPERIMENTAL OSTEOARTHRITIS IN RABBITS

B. Grigolo, G. Desando, C. Cavallo, M. Fini, N. Nicoli Aldini, R. Giardino, A. Facchini
Istituti Ortopedici Rizzoli, Bologna, Italy

Purpose: Autologous chondrocytes transplantation (ACI) has becoming a more diffuse therapeutical strategy in the repair of damaged cartilage in clinical practice. The data obtained at long-term follow-up showed good clinical results together with the formation of a new tissue with many hyaline features even if long time is required for a complete regeneration. The improvement of the technique is mainly due to the use of suitable scaffolds able to fix the cells into the defect site permitting their proliferation, maintainance of the chondrocyte phenotype and a easier surgical procedure. However, until now the indication for ACI are symptomatic full-thickness chondral injuries or osteochondritis dissecans and the treatment requires the harvest of healthy cartilage tissue and a cell expansion phase in vitro. In order to find a new approach to treat patients with osteoarthritis (OA) suffering for early degenerative lesions to hyaline cartilage, the aim of